

WE CLAIM:

1. A method for screening, diagnosis or prognosis of Alzheimer's disease in a mammal, for identifying a mammal at risk of developing Alzheimer's disease, and/or for monitoring the effect of therapy administered to a mammal having

5 Alzheimer's disease, said method comprising:

(a) analyzing a test sample of cerebrospinal fluid from the mammal by two dimensional electrophoresis to generate a two-dimensional array of one or more of the following Alzheimer's Disease-Associated Features (AFs): AF-1, AF-2, AF-3, AF-4, AF-5, AF-6, AF-7, AF-8, AF-9, AF-10, AF-13, AF-14, AF-15, AF-16, AF-17, AF-18, AF-19, AF-20, AF-21, AF-22, AF-23, AF-24, AF-25, AF-26, AF-27, AF-28, AF-29, AF-30, AF-31, AF-32, AF-33, AF-34, AF-35, AF-36, AF-37, AF-38, AF-39, AF-40, AF-41, AF-42, AF-43, AF-44, AF-45, AF-46, AF-47, AF-48, AF-49, AF-50, AF-51, AF-52, AF-53, AF-54, AF-55, AF-56, AF-57, AF-58, AF-59, AF-60, AF-61, AF-62, AF-63, AF-64, AF-65, AF-66, AF-67, AF-68, AF-69, AF-70, AF-71, AF-72, AF-73, AF-74, AF-75, AF-76, AF-77, AF-78, AF-79, AF-80, AF-81, AF-82, AF-83, AF-84, AF-85, AF-86, AF-87, AF-88, AF-89, AF-90, AF-91, AF-92, AF-93, AF-94, AF-95, AF-96, AF-98, AF-99, AF-100, AF-101, AF-102, AF-103, AF-104, AF-105, AF-107, AF-108, AF-110, AF-111, AF-112, AF-114, AF-115, AF-116, AF-117, AF-118, AF-119, AF-121, AF-122, AF-123, AF-124, AF-125, AF-126, AF-127, AF-128, AF-129, AF-130, AF-131, AF-132, AF-133, AF-134, AF-137, AF-139, AF-140, AF-141, AF-142, AF-143, AF-144, AF-145, AF-146, AF-147, AF-148, AF-149, AF-150, AF-151, AF-152, AF-153, AF-154, AF-155, AF-156, AF-157, AF-159, AF-160, AF-161, AF-162, AF-163, AF-164, AF-165, AF-166, AF-167, AF-168, AF-169, AF-170, AF-171, AF-172, AF-173, AF-174, AF-175, AF-176, AF-177, AF-178, AF-179, AF-180, AF-181, AF-182, AF-183, AF-184, AF-185, AF-186, AF-187, AF-188, AF-189, AF-190, AF-191, or AF-191; and

(b) comparing the abundance of each chosen feature in the test sample with the abundance of that chosen feature in body fluid from one or more persons free from Alzheimer's disease, or with a previously determined reference range for that feature in subjects free from Alzheimer's disease, or with the abundance at least one Expression Reference Feature (ERF) in the test sample.

2. The method of claim 1, wherein said method is for screening or diagnosis of Alzheimer's disease and the relative abundance of at least one chosen feature correlates with the presence or absence of Alzheimer's disease.

5 3. The method of claim 1, wherein said method is for monitoring the effect of therapy administered to a subject having Alzheimer's disease and the relative abundance of at least one chosen feature correlates with the severity of Alzheimer's disease.

10 4. A method for screening, diagnosis or prognosis of Alzheimer's disease in a mammal for identifying a mammal at risk of developing Alzheimer's disease, or for monitoring the effect of therapy administered to a mammal having Alzheimer's disease, said method comprising;

15 (a) quantitatively detecting, in a sample of cerebrospinal fluid from the mammal, at least one of the following Alzheimer's Disease-Associated Protein Isoforms (APIs): API-1, API-2, API-3, API-4, API-5, API-6, API-7, API-8, API-9, API-10, API-14, API-15, API-16, API-17, API-18, API-19, API-20, API-22, API-23, API-24, API-25, API-26, API-27, API-28, API-30, API-33, API-34, API-35, API-36, API-37, API-38, API-39, API-40, API-41, API-42, API-43, API-44, API-45, API-46, API-47, API-48, API-49, API-50, API-51, API-52, API-53, API-54, API-55, API-56, API-57, API-58, API-59, API-60, API-61, API-62, API-63, API-64, API-65, API-66, API-67, API-68, API-69, API-70, API-71, API-72, API-73, API-74, API-75, API-76, API-77, API-78, API-79, API-80, API-81, API-82, API-83, API-84, API-85, API-86, API-88, API-89, API-90, API-91, API-92, API-93, API-95, API-97, API-98, API-99, API-101, API-102, API-103, API-104, API-107, API-108, API-111, API-112, API-113, API-114, API-116, API-118, API-119, API-120, API-121, API-122, API-123, API-124, API-125, API-126, API-127, API-128, API-130, API-131, API-132, API-134, API-135, API-136, API-137, API-138, API-139, API-140, API-141, API-142, API-143, API-144, API-145, API-146, API-147, API-148, API-149, API-150, API-151, API-152, API-153, API-155, API-158, API-

159, API-160, API-161, API-162, API-163, API-165, API-166, API-
167, API-168, API-169, API-170, API-171, API-172, API-173, API-
174, API-175, API-176, API-177, API-178, API-179, API-180, API-
181, API-182, API-183, API-184, API-185, API-186, API-187, API-
188, API-189, API-190, API-191, API-192, API-194, API-196, API-
197, API-198, API-199, API-200, API-201, API-202, API-210, API-
214, API-215, API-217, API-219, API-220, API-221, API-222, API-
223, API-224, API-225, API-232, API-233, API-234, API-237, API-
238, API-239, API-240, API-241, API-242, API-243, API-244, API-
245, API-246, API-247, or API-248; and

10 (b) comparing the level or amount of said isoform or isoforms detected in
step (a) with a control.

5. The method according to claim 4, wherein the step of quantitatively

15 detecting comprises testing at least one aliquot of the sample, said testing comprising:

(a) contacting the aliquot with an antibody that is immunospecific for a
preselected API;
(b) quantitatively measuring any binding that has occurred between the
antibody and at least one species in the aliquot; and
20 (c) comparing the results of step (b) to a control.

6. The method according to claim 5, wherein the antibody is a monoclonal
antibody.

25 7. The method according to Claim 5, wherein the antibody is chimeric.

8. The method according to claim 5, wherein the step of quantitatively detecting comprises testing a plurality of aliquots with a plurality of antibodies for quantitative detection of a plurality of preselected APIs.

5 9. The method according to claim 8, wherein the antibodies are monoclonal antibodies.

10. The method according to claim 8, wherein the antibodies are chimeric.

11. A preparation comprising at least one of the following isolated Alzheimer's Disease-Associated Protein Isoform (API) said API selected from API-1, API-2, API-3, API-4, API-5, API-6, API-7, API-8, API-9, API-10, API-14, API-15, API-16, API-17, API-18, API-19, API-20, API-22, API-23, API-24, API-25, API-26, API-27, API-28, API-30, API-33, API-34, API-35, API-36, API-37, API-38, API-39, API-40, API-41, API-42, API-43, API-44, API-45, API-46, API-47, API-48, API-49, API-50, API-51, API-52, API-53, API-54, API-55, API-56, API-57, API-58, API-59, API-60, API-61, API-62, API-63, API-64, API-65, API-66, API-67, API-68, API-69, API-70, API-71, API-72, API-73, API-74, API-75, API-76, API-77, API-78, API-79, API-80, API-81, API-82, API-83, API-84, API-85, API-86, API-88, API-89, API-90, API-91, API-92, API-93, API-95, API-97, API-98, API-99, API-101, API-102, API-103, API-104, API-107, API-108, API-111, API-112, API-113, API-114, API-116, API-118, API-119, API-120, API-121, API-122, API-123, API-124, API-125, API-126, API-127, API-128, API-130, API-131, API-132, API-134, API-135, API-136, API-137, API-138, API-139, API-140, API-141, API-142, API-143, API-144, API-145, API-146, API-147, API-148, API-149, API-150, API-151, API-152, API-153, API-155, API-158, API-159, API-160, API-161, API-162, API-163, API-165, API-166, API-167, API-168, API-169, API-170, API-171, API-172, API-173, API-174, API-175, API-176, API-177, API-178, API-179, API-180, API-181, API-182, API-183, API-184, API-185, API-186, API-187, API-188, API-189, API-190, API-191, API-192, API-194, API-196, API-197, API-198, API-199, API-200, API-201, API-202, API-210, API-214, API-215, API-217, API-219, API-220, API-221, API-222, API-223, API-224, API-225, API-232, API-233, API-234, API-237, API-238, API-239, API-240, API-241, API-242, API-243, API-244, API-245, API-246, API-247, or API-248.

12. A kit comprising the preparation of claim 11, other reagents, and directions for use.

30 13. The kit of Claim 12 comprising a plurality of said preparations.

14. A preparation comprising an isolated human protein, said protein comprising a tryptic digest peptide having the following partial sequence as determined by mass spectrometry: PGLGM.

5 15. A preparation comprising an isolated human protein, said protein comprising a tryptic digest peptide having the following partial sequence as determined by mass spectroscopy: GPLGM.

10 16. A preparation comprising an isolated human protein, said protein comprising a tryptic digest peptide having the following partial sequence as determined by mass spectroscopy: PGLGF.

15 17. A preparation comprising an isolated human protein, said protein comprising a tryptic digest peptide having the following partial sequence as determined by mass spectroscopy: GPLGF.

20 18. A preparation comprising an isolated human protein, said protein comprising a tryptic digest peptide having the following partial sequence as determined by mass spectrometry: PGIGM.

19. A preparation comprising an isolated human protein, said protein comprising a tryptic digest peptide having the following partial sequence as determined by mass spectroscopy: GPIGM.

25 20. A preparation comprising an isolated human protein, said protein comprising a tryptic digest peptide having the following partial sequence as determined by mass spectroscopy: PGIGF.

21. A preparation comprising an isolated human protein, said protein comprising a tryptic digest peptide having the following partial sequence as determined by mass spectroscopy: GPIGF.

5 22. The preparation according to any one of claims 14, 15, 16, 17, 18, 19, 20 or 21, wherein the tryptic digest peptide has a mass of 1546.73 Da, and an N-terminal mass of 0 Da, and a C-terminal mass of 1076.63 Da, said masses having an error of measurement of 100 parts-per-million or less.

10 23. The preparation according to any one of claims 14, 15, 16, 17, 18, 19, 20 or 21, wherein the protein further comprising a tryptic digest peptide having the following partial sequence as determined by mass spectrometry: HQV.

15 24. The preparation according to any one of claims 14, 15, 16, 17, 18, 19, 20 or 21, wherein the protein further comprising a tryptic digest peptide having the following partial sequence as determined by mass spectrometry: HQV, wherein the tryptic digest peptide has a mass of 1096.56 Da, and an N-terminal mass of 0 Da, and a C-terminal mass of 733.50 Da, said masses having an error of measurement of 100 parts-per-million or less.

20 25. A preparation comprising an isolated human protein, said protein comprising a tryptic digest peptide having the following partial sequence as determined by mass spectroscopy: HQV.

25 26. The preparation according to claim 25 wherein the tryptic digest peptide has a mass of 1096.56 Da, and an N-terminal mass of 0 Da, and a C-terminal mass of 733.50 Da, said masses having an error of measurement of 100 parts-per-million or less.

27. The preparation according to any one of claims 14, 15, 16, 17, 18, 19, 20,¹
21, 25 or 26, wherein the protein has an isoelectric point (pI) of about 6.80 and an/
apparent molecular weight (MW) of about 18,741.

5 28. An isolated nucleic acid molecule that hybridizes to a nucleotide sequence
encoding API-111, a nucleotide sequence encoding API-112, or their complements.

10 29. An isolated nucleic acid molecule that hybridizes to a nucleotide sequence
encoding at least 10 consecutive amino acids of API-111 a nucleotide sequence
encoding at least 10 consecutive amino acids of API-112, or their complements.

30. A vector comprising the nucleic acid molecule of claim 28 or 29.

15 31. A host cell comprising the vector of claim 28.

32. A host cell genetically engineered to express the nucleic acid molecule of
claim 28 or 29.

20 33. An isolated nucleic acid molecule that hybridizes under highly stringent
conditions or moderately stringent conditions to the following nucleic acid sequence:
CCNGGNYTNNGGNATG.

25 34. An isolated nucleic acid molecule that hybridizes under highly stringent
conditions or moderately stringent conditions to the following nucleic acid sequence:
GGNCCNYTNNGGNATG.

35. An isolated nucleic acid molecule that hybridizes under highly stringent

conditions or moderately stringent conditions to the following nucleic acid sequence:
CCNGGNYTNGGNTTY. 1

36. An isolated nucleic acid molecule that hybridizes under highly stringent
5 conditions or moderately stringent conditions to the following nucleic acid sequence:
GGNCCNYTNGGNTTY.

37. An isolated nucleic acid molecule that hybridizes under highly stringent
conditions or moderately stringent conditions to the following nucleic acid sequence:
10 CCNGGNATHGGNATG.

38. An isolated nucleic acid molecule that hybridizes under highly stringent
conditions or moderately stringent conditions to the following nucleic acid sequence:
CCNGGNATHGGNTTY.

15 39. An isolated nucleic acid molecule that hybridizes under highly stringent
conditions or moderately stringent conditions to the following nucleic acid sequence:
GGNCCNATHGGNATG.

20 40. An isolated nucleic acid molecule that hybridizes under highly stringent
conditions or moderately stringent conditions to the following nucleic acid sequence:
GGNCCNATHGGNTTY.

25 41. The isolated nucleic acid molecule according to any one of claims 33, 34,
35, 36, 37, 38, 39, or 40, wherein the nucleic acid also hybridizes under highly
stringent conditions or moderately stringent conditions to the following nucleic acid
sequence:

CAYCARGTN.

42. An isolated nucleic acid molecule that hybridizes under highly stringent conditions or moderately stringent conditions to the following nucleic acid sequence:
CCCGGCCTGGGCATG.

5 43. An isolated nucleic acid molecule that hybridizes under highly stringent conditions or moderately stringent conditions to the following nucleic acid sequence:
GGCCCCCTGGGCATG.

10 44. An isolated nucleic acid molecule that hybridizes under highly stringent conditions or moderately stringent conditions to the following nucleic acid sequence:
CCCGGCCTGGGCTTC.

15 45. An isolated nucleic acid molecule that hybridizes under highly stringent conditions or moderately stringent conditions to the following nucleic acid sequence:
GGCCCCCTGGGCTTC.

20 46. An isolated nucleic acid molecule that hybridizes under highly stringent conditions or moderately stringent conditions to the following nucleic acid sequence:
CCCGGCATCGGCATG.

47. An isolated nucleic acid molecule that hybridizes under highly stringent conditions or moderately stringent conditions to the following nucleic acid sequence:
CCCGGCATCGGCTTC.

25 48. An isolated nucleic acid molecule that hybridizes under highly stringent conditions or moderately stringent conditions to the following nucleic acid sequence:
GGCCCCATCGGCATG.

49. An isolated nucleic acid molecule that hybridizes under highly stringent conditions or moderately stringent conditions to the following nucleic acid sequence:
GGCCCCATCGGCTTC.

50. The isolated nucleic acid molecule according to any one of claims 42, 43, 44, 45, 46, 47, 48 or 49, wherein the nucleic acid also hybridizes under highly stringent conditions or moderately stringent conditions to the following nucleic acid sequence:

CACCAGGTG.

10

*att
B1*